

**THE SPATIAL GAMES: INVESTIGATING THE SPATIAL DISTRIBUTION OF ANAX AND SELECT  
PREY AS A FUNCTION OF PREDATOR-PREY INTERACTIONS**

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INVESTIGATING THE SPATIAL DISTRIBUTION OF *ANAX* AND SELECT PREY AS A  
FUNCTION OF PREDATOR-PREY INTERACTIONS**

**ABSTRACT**

Our study investigated the spatial distribution and movement behavior of predatory dragonfly larvae (*Anax*) and of two prey types: mosquito larvae and amphipods. Predator-prey interactions have important consequences for the population dynamics of both predator and prey groups and these interactions can shape community structure. We measured behavior of each prey type in the presence of the *Anax* predator and the behavior of the predator in the presence of these alternative prey types. Observations were made in five-gallon aquaria where a grid pattern allowed us to track the number of moves made by individuals. We compiled data from ten, one hour trials for each predator-prey combination (*Anax* + amphipods and *Anax* + mosquito larvae). Prey species differed in their behavior. Mosquito larvae spent more time near the water's surface, were more likely to utilize the artificial vegetation, and were more active than amphipods. On the other hand, amphipods utilized full range of the aquarium and had a greater number of moves than mosquito larvae. *Anax* behavior was significantly different in the two prey treatments. *Anax* spent more time in the top portion of the aquarium during the mosquito treatments, utilized the artificial vegetation more in the amphipod treatments, had a greater number of moves in the amphipod treatments, and ate more amphipods than mosquito larvae. Our results indicate that *Anax* change their behavior based on prey type.

## INTRODUCTION

Predator-prey interactions are a common subject of study and research in the ecological world. Predator-prey interactions have important consequences for the population dynamics of both groups and these interactions can shape community structure (Wellborn et al. 1996). Previous experiments have examined the predator-prey interactions shape the distributions of both trophic levels with the common pattern being that predators will aggregate to and concentrate their efforts upon large densities of prey (Hassell 1978; Cowie and Krebs 1979) and prey will move into areas with a lower amount of predation risk (Stein 1979; Morse 1980; Sih 1982a; Preisser et al. 2005). We want to understand the mechanics of spatial distribution so we can understand who is driving these distribution patterns and what this means for population dynamics and community structure. In our investigation, we will explore the following questions:

- 1) What is the driving force for spatial distribution?
- 2) Who is driving spatial distribution: predators or prey?
- 3) What are the physical differences between prey types, in this case, amphipods and mosquitoes, and which prey type is likely to be most attractive to dragonfly larvae?

We hypothesized that the spatial distribution of predators is driven by resource density and the spatial distribution is driven by both resource density and predation avoidance. In models discussed by Hammond and colleagues (2007) predators prefer areas with higher prey densities and prey avoid areas with higher predation risk (Stephens and Krebs 1986, Lima and Dill 1990, Sih 1998). This results in a fundamental tension between these distributions with predators pursuing and prey trying to avoid predators. However, almost all of those studies focused only

on either predators or prey and don't investigate the spatial distribution of both together, they also only look at one prey type but different prey may produce very different patterns. These models provide insight on behavior of both trophic levels in the presence of resources. We can use these to provide context for interpreting your data but not directly as controls. Hammond et al. (2007) notes that in these models predators choose patches based on their expected predation success. Their success is usually a function of encounter rates with prey and competition with other predators. Thus, predators should favor patches with aggregations of prey. And prey should also shift their spatial distribution in response in order to avoid predators.

We predict that the distributions of predators and prey will result from an interaction between these groups rather than one trophic level being the sole driver of their joint distributions. Predators shift their spatial distribution based on resources and food availability. While prey are focused on their escape from predators, predators are focused on foraging. Predators aren't pressed by an immediate life-or-death situation so they can be relatively less responsive than prey. On the other hand, prey must choose their movements based on predator movements in order for them to survive to see another day. Although it is true that predators could afford to skip a meal, without access to their prey they will starve. This means, that in order to survive, dragonfly larvae are dependent on the location of their prey. Studies show that dragonfly larvae will shift their habitat use based on where their prey reside (Hammond et al. 2007). We predict in our experiment that both types of prey will have an effect on the location of the predators. The prey's body size can be used as a proxy for the energetic gains that each prey type has for the predator. Predators are expected to prefer more profitable prey. Mosquito larvae are approximately four to five times larger than the immature, juvenile amphipods and two to three times larger than the mature, adult amphipods. We would expect the dragonfly larvae to spend

more time going after the mosquito larvae based on this estimate of body size since the mosquito larvae would be more beneficial in terms of food size. Consequently, we expect to find a difference in the dragonfly larvae's foraging behavior between the two prey types.

We expect the predators to have significantly different spatial responses to the two prey types. They will have a preference for the mosquitoes because they are a more profitable catch when their body size is considered. The fact that mosquito larvae are surface breathers should play a big role in their spatial distribution in the tanks. The mosquitoes will most likely reside in the top portion of the tank and their spatial distribution will be limited. The dragonfly larvae should spend more time in the top portion of the tank during the mosquito treatment. During the amphipod trials, the amphipods will not be limited to the top portion of the tank and should have a larger spatial distribution across the whole tank. The spatial distribution of *Anax* during the amphipod trials is therefore expected to be more variable.

## **METHODS**

### *Experimental Setup*

We used 8 five-gallon aquariums as observation tanks for our experimentation. Grids were drawn onto the front and sides of the aquarium. These grids were used as quadrants to determine the movement and spatial distribution of the predator and prey individuals. The front panel was sectioned into six squares, three on the top (numbered #1-3 from left to right) and three on the bottom (numbered #4-6 from left to right). A vertical line was drawn on the sides of the tank to separate the front from the back. These aquariums were stocked with three cords of artificial vegetation made out of frayed yellow polypropylene rope. The rope cords were tied to metal nuts to prevent the vegetation from moving around the aquarium. These were spaced so

that there was one chord of artificial vegetation in each column. The tanks were filled with tap water that had been aged for about a week.

#### *Collection and Maintenance of Anax*

We collected 43 dragonfly larvae in the genus *Anax* (Odonata:Anisoptera:Aeshnidae) from a pond near the Cal Poly SLO campus. These individuals were housed in small plastic fish aquariums filled with aged water. Each container was supplied with pebbles and pieces of screening for the *Anax* individuals to provide them with a substrate they could move easily on. The water in these housing units was changed approximately every two weeks. We fed the *Anax* with zooplankton on a schedule of Tuesday, Thursday and either Saturday or Sunday. If their size dictated a greater food amount, we would supplement their feeding with damselflies as needed. If the *Anax* larva's wing pads looked puffy or were starting to, they were provided with damselfly larvae as food.

#### *Collection and Maintenance of Mosquito larvae and Amphipods*

Amphipods were collected from the same pond as the *Anax*. Mosquito larvae were collected by leaving containers of water outside and collecting the offspring of the mosquitoes that laid their eggs there. The amphipods and mosquito larvae were housed separately in aggregate containers of aged water. We added small pellets of rabbit food to the amphipod container to help sustain the population. The mosquito larvae were not fed.

### *Behavioral Observations*

We observed predator and prey interactions between the *Anax* larvae and amphipod and mosquito larvae with *Anax* as our predators and the amphipods and mosquitoes as our two prey types and thus, our two treatments. We chose the eight *Anax* individuals for each trial by using an online random number generator on the website RANDOM.org. The *Anax* individuals were taken from their housing units and put into the experimental tanks. We allowed the *Anax* to acclimate to their new environment for ten minutes. Then three prey individuals, either mosquito or amphipod individuals, were added to the tanks as well and given an acclimation period of five minutes. After this five minute interval, we began our behavioral observations. We recorded behavioral data at five minute intervals. We observed what square the *Anax* and its prey were in, whether or not they were on the rope, and whether or not they were active at the time they were observed. Data was collected from about ten trials of each treatment.

### *Data Analysis*

We combining the trials of our predator and prey behavioral data and used t-tests to compare predator behavior with the different prey types and prey behavior in the presence of *Anax*. Three t-tests were performed to compare *Anax* behavior in the presence of amphipods to their behavior in the presence of mosquito larvae. These behaviors include number of moves made in each prey treatment, the proportion of time spent at the top of the aquarium, and proportion of time spent on artificial vegetation. This same analysis was done for the amphipods and mosquito larvae in the presence of *Anax* with the proportion of activity of prey individuals included. In the case of the *Anax* larvae, we also recorded and analyzed the mean number of prey eaten. We ended up dropping the activity of our *Anax* predators from the analysis. The reason as

to why we drop activity will be further explored in our discussion. We also performed ANOVA tests on trial number to determine whether there was a significant difference in variability between trials.

## RESULTS

### *Predator Results: Predation Rates and Behavior*

The possible effect of trial on behavioral measures was determined using an ANOVA test. The behavioral measure “activity” was eliminated from our comparisons because there was a significant effect of trial in recording these data (ANOVA: Amphipods:  $F_{1,9} = 4.9$ ,  $p < 0.001$ , Mosquitoes:  $F_{1,9} = 5.79$ ,  $p = 0.05$ ). No other behaviors differed significantly between trials (all  $p > 0.05$ ). The number of prey eaten was compared for the two treatments using a t-test. Significantly more amphipods were eaten by *Anax* than mosquitoes ( $t = 12.015$ ,  $p < 0.001$ ,  $df = 152$ , Figure 1). The percentage of time the predator spent on the top portion of the tank was compared between the two treatments using a t-test. *Anax* spent significantly more time near the water surface in the mosquito treatment relative to the amphipod treatment ( $t = -8.961$ ,  $p < 0.001$ ,  $df = 152$ , Figure 2). The proportion of time the *Anax* spent on rope, or artificial vegetation, was compared between the two treatments using a t-test. The predators were on artificial vegetation more often in the amphipod treatment than in the mosquito treatment ( $t = 10.627$ ,  $p < 0.001$ ,  $df = 152$ , Figure 3). The average number of moves made by the *Anax* in each treatment was compared using a t-test. *Anax* were more likely to move in the amphipod treatment than the mosquito treatment ( $t = 15.462$ ,  $p < 0.001$ ,  $df = 152$ , Figure 4).



### *Prey Results: Behavior*

The proportion of time each prey spent on the top portion of the tank was compared using a t-test. The mosquito larvae spent more time on the top of the tank than the amphipods ( $t = -3.184$ ,  $p = 0.002$ ,  $df = 152$ , Figure 5). The proportion of time each prey spent on the artificial vegetation was compared using a t-test comparison. The mosquito larvae spent more time on the rope than the amphipods ( $t = -5.143$ ,  $p < 0.001$ ,  $df = 152$  Figure 6). The average number of moves of the two types of prey was compared using a t-test. There was a significant difference between amphipods and mosquitoes in the number of moves made during the trials ( $t = 2.715$ ,  $p = 0.007$ ,  $df = 152$ , Figure 7). The activity level of each prey species was compared using a t-test. The mosquito larvae had an overall higher activity level than the amphipods ( $t = -5.391$ ,  $p < 0.001$ ,  $df = 152$ , Figure 8).

## **DISCUSSION**

*Anax* larvae spent more time at the top of the tank in the mosquito treatment compared to the amphipod treatment. In the amphipod treatment, *Anax* moved more often and spent more time on artificial vegetation. Despite their greater size, mosquitoes were consumed less frequently than amphipods by *Anax* during these trials. Prey species differed in their behavior. Mosquitoes had higher activity levels, spent more time on the top portion of the tank and spent more time on the artificial vegetation than the amphipods. However, the amphipods moved more frequently than the mosquitoes.

It appears that *Anax* are actively foraging on mosquito larvae but their lessened overall movement in the amphipod treatment indicates the *Anax* are not actively pursuing the amphipods. The number of moves made by *Anax* was significantly higher in the amphipod treatment. This may be attributable to the fact that the mosquito larvae breathe at the surface of

the water and are therefore more restricted to the upper portion of the tank. This could result in a lower number of moves by the *Anax* because mosquito larvae are found in a smaller fraction of the tank's total area. The spatial distribution of the mosquitoes also restricts the *Anax* who are tracking them to the top portion of the tank, a pattern we observed in our results. Because the amphipods are not surface breathers, they are not limited spatially and have full range of the aquarium. From our data, it is clear that the *Anax* larvae are shifting their spatial behavior differently for different prey types. *Anax* move more often when hunting for amphipods because amphipods move more often. And *Anax* move less often and spent more time in the top portion of the aquarium in order to track mosquito larvae that move less often and spend most of their time at the surface.

It was observed during the mosquito larvae treatment trials that there were several instances where the *Anax* larvae were actively attempting to capture the mosquito larvae. The *Anax* larvae were not as successful at capturing mosquito larvae as they were at catching amphipods. During the ten rounds of trials, only eighteen mosquito larvae were successfully consumed while eighty-three amphipods were consumed. The mosquito larvae had a significantly higher mean activity when compared to amphipods. This is interesting because we would expect that a higher mean activity would also lead to an increase in the possibility of encountering a predator since it would make them more visible to a motion-sensitive predator (Werner and Anholt 1993). We found that activity was not an important predictor of vulnerability to predation since so few of the mosquito individuals in the trials were caught by *Anax*.

A number of factors may explain why the mosquito larvae had lower mortality rates than amphipods. First, mosquitoes spent a significant amount of time in the top portion of the

aquarium. Although *Anax* in the mosquito treatments spent a significantly greater amount of time in the top portion of the aquarium as a result, *Anax* still moved very little infrequently in the mosquito treatment trials. Mosquito larvae also spent more time on the artificial vegetation. We predict that the rope provides a refuge from the *Anax* larvae since *Anax* in the mosquito trials spent considerably less time on the rope. With the combination of high activity and an effective hiding strategy, mosquito larvae greatly increased their ability to successfully evade capture by *Anax*.

In the trials that they were observed in, amphipods were quick in their movements and many tended to run circles around the *Anax*. When compared to the mosquitoes, the amphipods were less active, but they moved more often. While activity may be an important factor, the number of movements appeared to be more relevant to predation risk. Although higher activity would cause prey individuals to be more noticeable to predators, a higher number of physical movements directly determines the spatial distance between a prey individual and their predator. We would expect that with significantly less activity and but only a slightly higher number of moves, there would be fewer amphipod mortalities since there would be a lower probability of encountering a predator. But in the case of amphipods, they were caught and eaten by *Anax* at a significantly higher frequency than mosquitoes. We evaluated reasons for why this may occur.

In some species of amphipod, in the genus *Hyalella*, their mating behavior includes a period of precopulatory mate guarding. The male grabs the female by her lower back segment and carries her around ventrally (Borowsky 1984). We observed the amphipod individuals doing this prior to the experimental observations and we did see individuals behaving in this manner at random intervals during the observations. When separate, amphipod individuals tended to circle back to the bottom corners of the aquariums as a group. We believe that this “circling back”

may be a remnant of the previous copulatory behavior. Male amphipod individuals may be attempting to continue with the mating behavior and circle back to that corner to proceed. Amphipods also spent significantly less time on the artificial vegetation and consequently, we more frequently out in the open than hidden. As a result of these factors, amphipods would be much easier for an *Anax* individual to catch. *Anax* would have time to observe, recognize this circling pattern that the amphipods perform and prepare an attack.

For the *Anax* analyses, we chose to drop activity. We believe dropping this measure is justified because, in relation to predators, it is a very subjective behavior and has an unclear interpretation. The activity behavior produced very different patterns than the other data and confused the interpretation of these results. For example, in the prey analyses, it is very easy to interpret what can be considered activity and what cannot. The amphipods and mosquito larvae would move obvious distances which could be accurately considered to be “activity”. *Anax* larvae, on the other hand, moved little overall and only moved a slightly significant amount more in the mosquito treatment. However, what could be considered to be categorized as movement was not consistent across the board. We considered swimming, walking, and crawling to count as “active” behavior and excluded grooming from the equation. But there were few instances that could be accurately defined as characterizing any of these behaviors. Thus, any movement, even the slight waving of appendages by *Anax* could be considered as active. Since we found that the interpretation of “activity” for *Anax* would be prone to error, it was dropped and we focused on number of moves to more accurately determine spatial distribution. Additionally the presence of a significant trial effect suggests that this measure was less consistent than the other measures of behavior analyzed here.

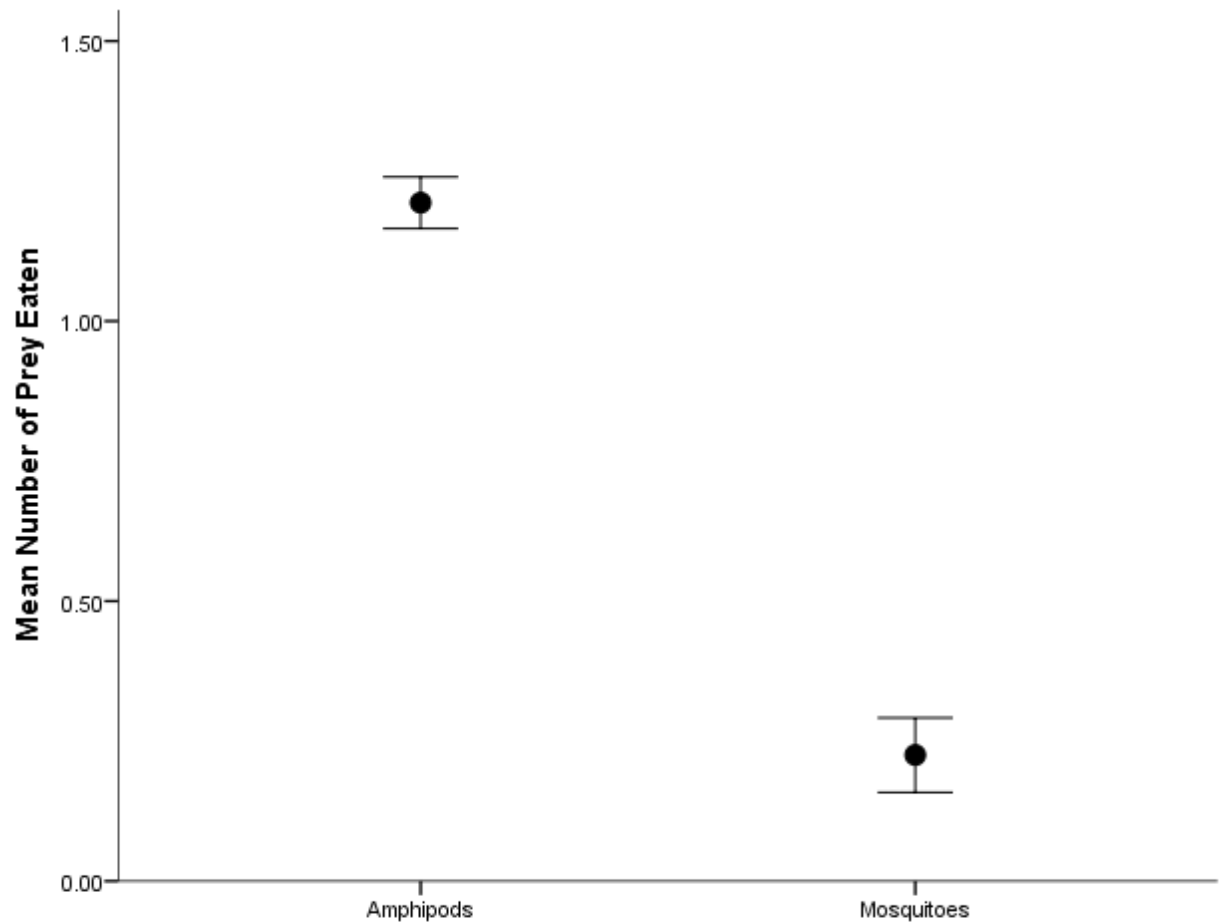
We do not have data on the behavior of *Anax* in the absence of prey and while these data would have been useful we believe that we can still make strong inferences about the effects of prey on predator behavior because foraging individuals are in a distinctly different state than non-foraging individuals. The activities of foraging individuals are influenced by the goal of catching their prey, while the activities of non-foraging individuals are not influenced by this factor. Non-foraging data from a separate study of larval dragonfly behavior that concluded that an individual's non-foraging and foraging activity levels were not significantly different. In this study, the activity levels of odonate predatory species were studied in the absence and presence of the prey, zooplankton. The study concluded that the predator behavior did not change in the presence of prey relative to their behavior in the absence of prey (Johansson 1992). Although this study differs from ours in species of dragonfly larvae studied and the prey type, these results suggest we can make inferences about behavior from the results we have. In our study, the observed individuals are in foraging mode. It is similar to comparing non-mating individuals and mating individuals in a study comparing different mating techniques. In studies that investigate the effects of variables on mating individuals, they focus on only mating individuals.

The first question we addressed in our experiment was to determine the driving force for spatial distribution of foraging dragonfly larvae. We can conclude that the spatial distribution of our predators was driven by resource density as we first predicted. Since we didn't actually have resource patches available for them, we can conclude that the spatial distribution of prey was largely driven by predator avoidance for mosquito larvae and mating behavior for amphipods. We also addressed the question of whether predators or prey are driving spatial distribution. From our results, we can conclude that the distribution did in fact result from a combination of interactions between both groups. Predators shifted their spatial distribution based on the

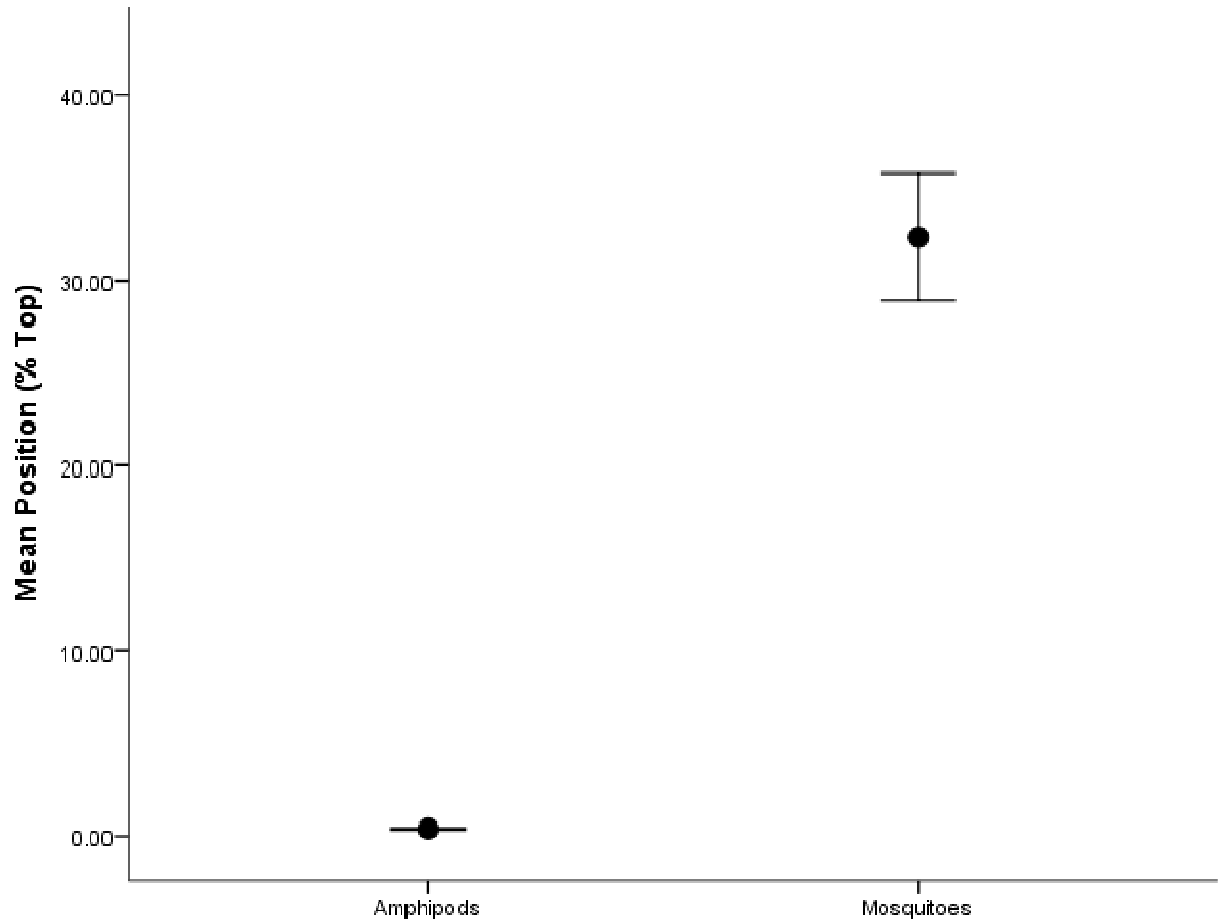
location of the prey, tracking mosquitoes toward the top of the tank. *Anax* also changed their behavior in other ways, moving around more in the presence of the amphipods that also moved more.

Finally, we addressed the question of which prey type is likely to be most attractive to dragonfly larvae. We initially predicted that the mosquito larvae would be more attractive to *Anax* based on the overall body size of the mosquito larvae. But since the *Anax* were largely unsuccessful in catching mosquito larvae, our answer to this question is inconclusive. We can only say that the reason that amphipods were caught more often was because they were more easily accessible to the *Anax*. We cannot necessarily say that they were caught more because they were more attractive to the *Anax*. After completing this experiment, we can conclude that there is no true winner in the Spatial Games.

## FIGURES

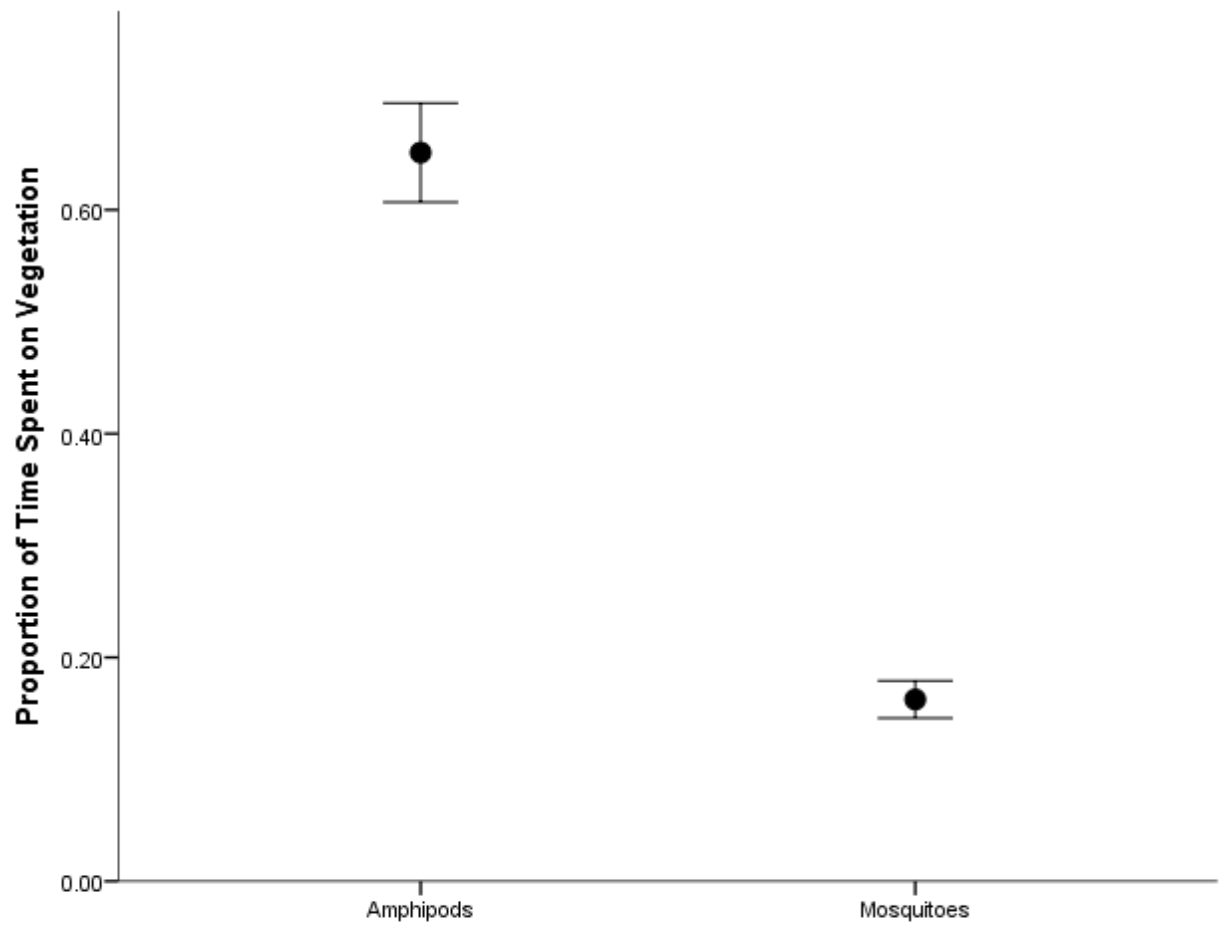


**Figure 1:** The mean number of prey eaten by dragonfly larvae in the amphipod and mosquito treatments. Dragonfly larvae consumed significantly more amphipods than mosquitoes. Error bars represent the standard error.

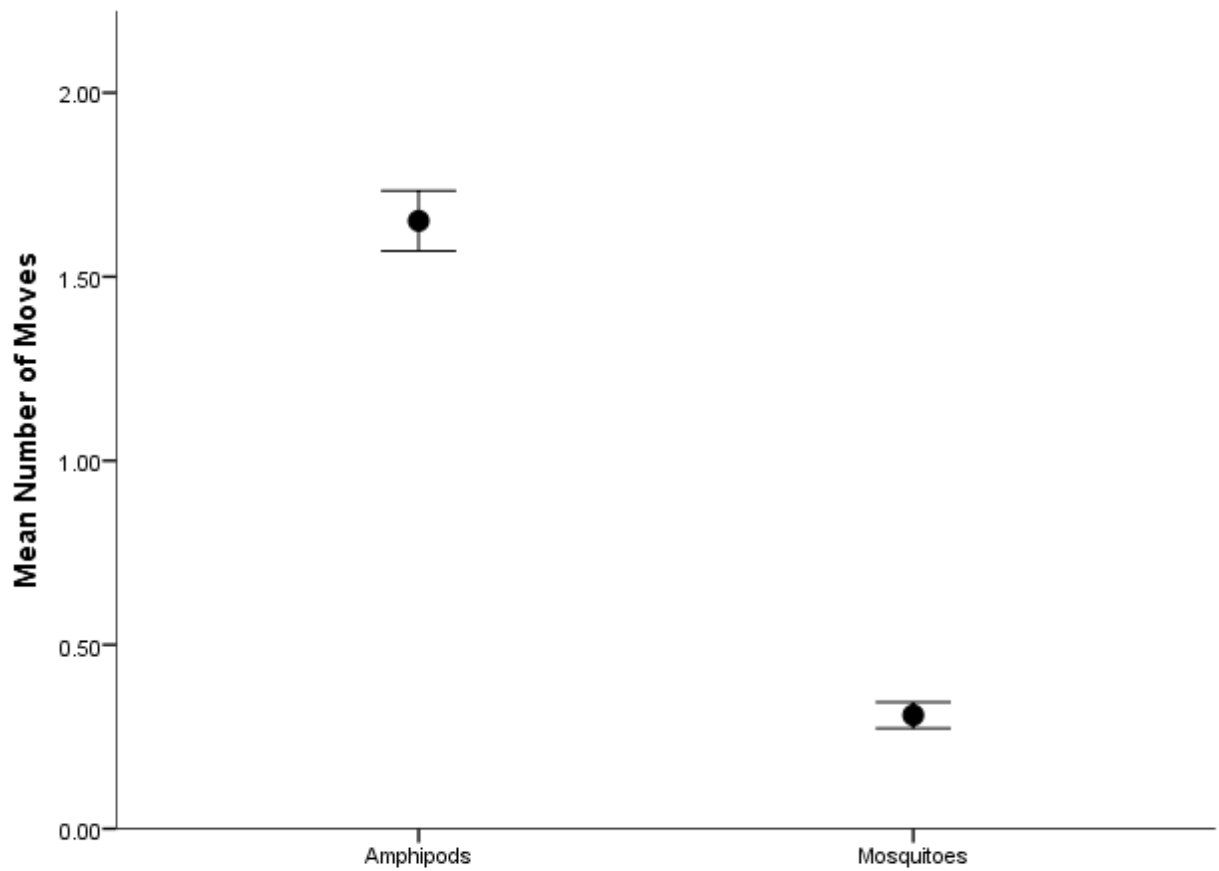


**Figure 2:** The mean frequency of time that dragonfly larvae in the amphipod and mosquito treatments spent in the top portion of the tank. Dragonfly larvae in the mosquito treatment spent significantly more time in the top half of the tank than in the amphipod treatment. Error bars represent the standard error.

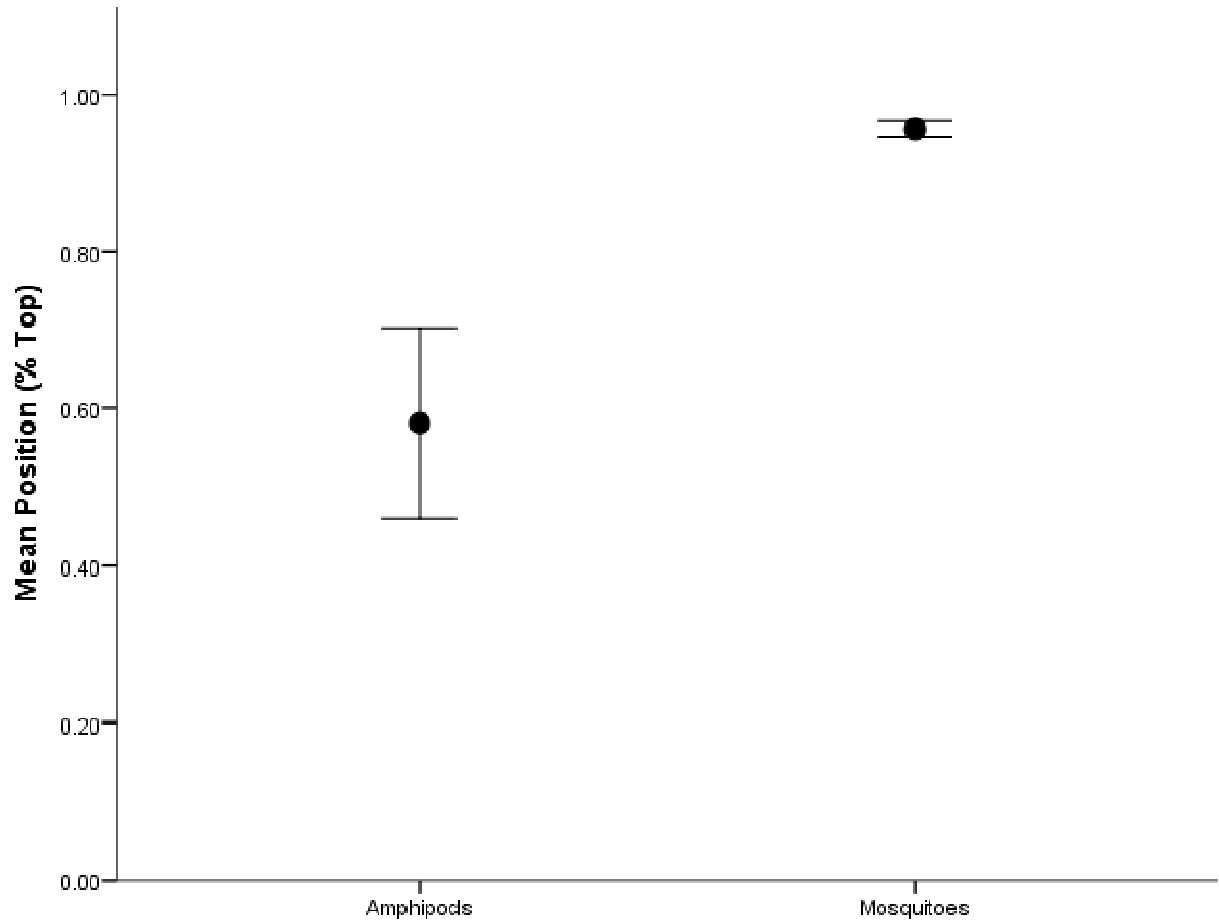




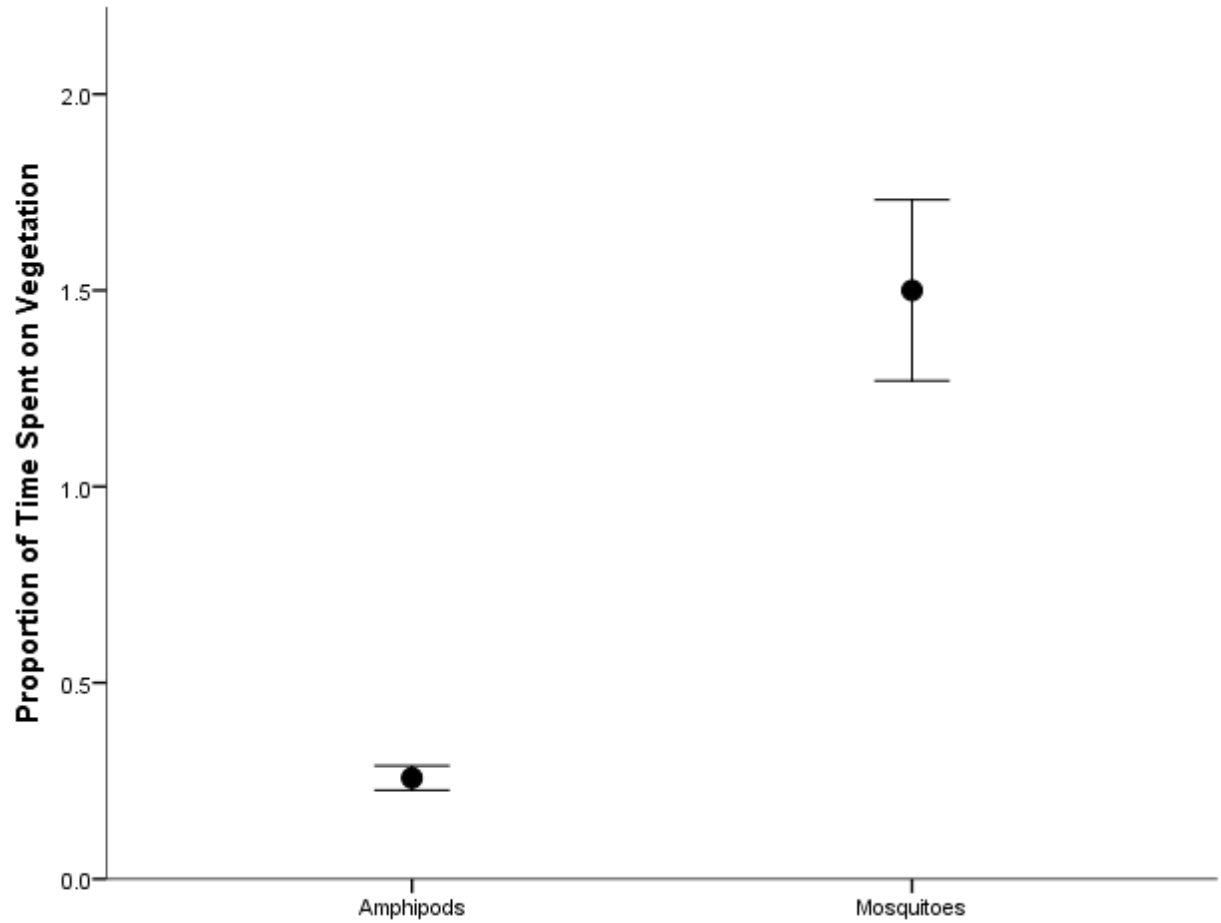
**Figure 3:** The mean proportion of time spent on the artificial vegetation by dragonfly larvae in the amphipod and mosquito treatments. The mean proportion of time spent on the rope by dragonfly larvae in the amphipod treatment is significantly higher than in the mosquito treatment. Error bars represent the standard error.



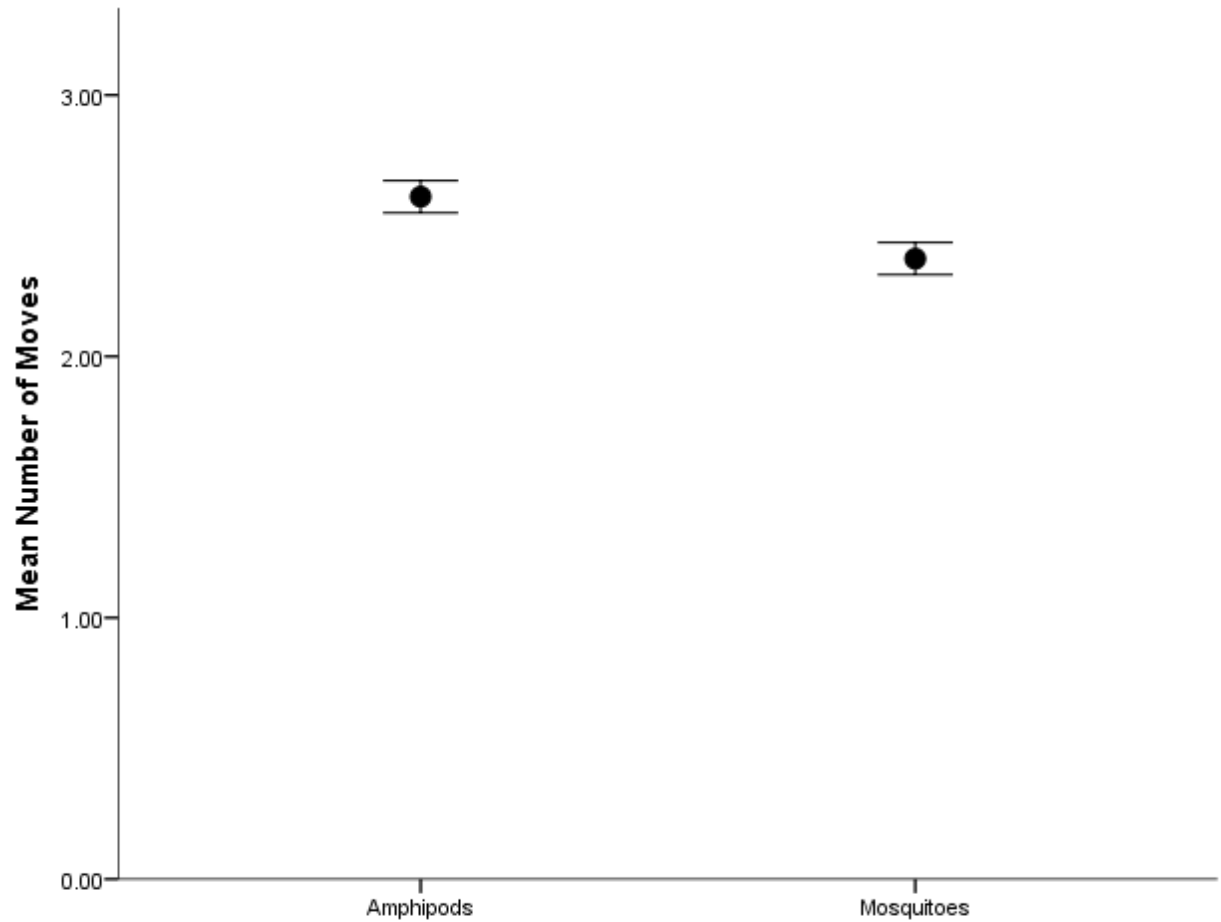
**Figure 4:** The mean number of moves by dragonfly larvae in the amphipod and mosquito treatments. The mean number of moves by dragonfly larvae in the amphipod treatment is significantly higher than in the mosquito treatment. Error bars represent the standard error.



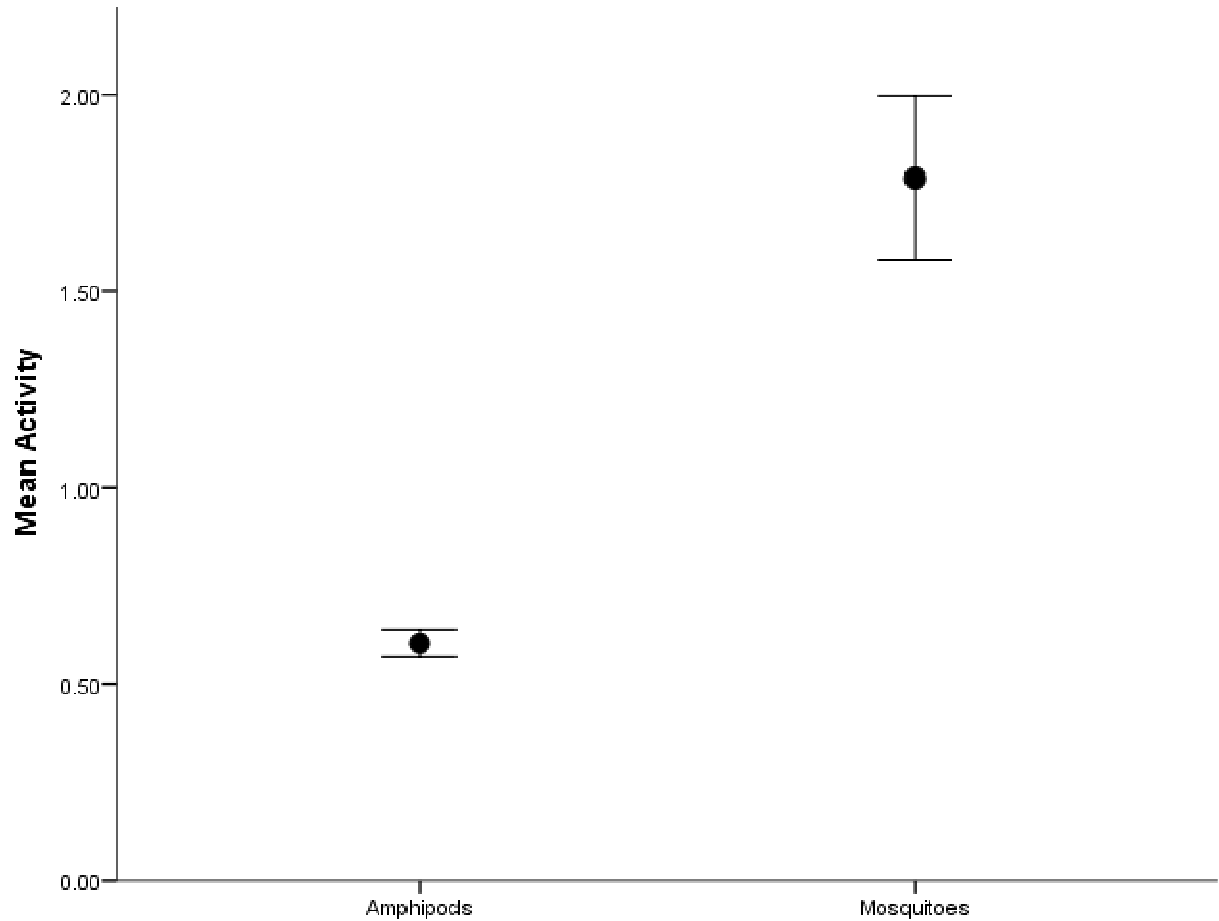
**Figure 5:** The mean frequency of time spent in the top portion of the tank prey species. Mosquitoes spent significantly more time in the top half of the tank than the amphipods. Error bars represent the standard error.



**Figure 6:** The proportion of time spent on the artificial vegetation by prey species. The mean proportion of time spent on the rope by mosquitoes is significantly higher than amphipods. Error bars represent the standard error.



**Figure 7:** The mean number of moves by prey species. There is significant difference between the mean number of moves for amphipods and mosquitoes. Amphipods have a slightly higher mean number of moves than mosquito larvae. Error bars represent the standard error.



**Figure 8:** The mean activity of prey species. The mean activity of mosquitoes is significantly higher in the amphipods. Error bars represent the standard error.

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